JOURNAL OF VIROLOGY, May 1999, p. 3951–3959 Vol. 73, No. 5
0022-538X/99/$04.00+0

Cellular Entry of Hantaviruses Which Cause Hemorrhagic Fever with Renal Syndrome Is Mediated by β_3 Integrins

IRINA N. GAVRILOVSKAYA,[1,2] ERIC J. BROWN,[3] MARK H. GINSBERG,[4] AND ERICH R. MACKOW[1,2,5]*

Department of Medicine[1] and Department of Molecular Genetics and Microbiology,[2] State University of New York at Stony Brook, Stony Brook, New York 11794; Washington University School of Medicine, St. Louis Missouri 63110[3]; Department of Vascular Biology, The Scripps Research Institute, La Jolla, California 92037[4]; and Northport Veterans Administration Medical Center, Northport, New York 11768[5]

Received 17 November 1998/Accepted 27 January 1999

Hantaviruses replicate primarily in the vascular endothelium and cause two human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In this report, we demonstrate that the cellular entry of HFRS-associated hantaviruses is facilitated by specific integrins expressed on platelets, endothelial cells, and macrophages. Infection of human umbilical vein endothelial cells and Vero E6 cells by the HFRS-causing hantaviruses Hantaan (HTN), Seoul (SEO), and Puumala (PUU) is inhibited by antibodies to $\alpha_v\beta_3$ integrins and by the integrin ligand vitronectin. The cellular entry of HTN, SEO, and PUU viruses, but not the nonpathogenic Prospect Hill (PH) hantavirus (i.e., a virus with no associated human disease), was also mediated by introducting recombinant $\alpha_{IIb}\beta_3$ or $\alpha_v\beta_3$ integrins into β_3-integrin-deficient CHO cells. In addition, PH infectivity was not inhibited by $\alpha_v\beta_3$-specific sera or vitronectin but was blocked by $\alpha_5\beta_1$-specific sera and the integrin ligand fibronectin. RGD tripeptides, which are required for many integrin-ligand interactions, are absent from all hantavirus G1 and G2 surface glycoproteins, and GRGDSP peptides did not inhibit hantavirus infectivity. Further, a mouse-human hybrid β_3 integrin-specific Fab fragment, c7E3 (ReoPro), also inhibited the infectivity of HTN, SEO, and PUU as well as HPS-associated hantaviruses, Sin Nombre (SN) and New York-1 (NY-1). These findings indicate that pathogenic HPS- and HFRS-causing hantaviruses enter cells via β_3 integrins, which are present on the surfaces of platelets, endothelial cells, and macrophages. Since β_3 integrins regulate vascular permeability and platelet function, these findings also correlate β_3 integrin usage with common elements of hantavirus pathogenesis.

Hantaviruses are enveloped viruses which define a unique genus within the *Bunyaviridae* and contain a tripartite negative-stranded RNA genome. The gene segments L, S, and M encode the viral RNA polymerase, the nucleocapsid protein (N), and two integral membrane surface glycoproteins (G1 and G2), respectively. Hantavirus glycoproteins are cotranslationally cleaved from a polyprotein precursor and define the highly ordered surface structure of the virion envelope (49).

Hantavirus infections are known to cause two human diseases, hemorrhagic fever with renal syndrome (HFRS) and a highly lethal (>40%) hantavirus pulmonary syndrome (HPS). In 1993, cases of adult respiratory distress in the southwestern United States led to the discovery of Sin Nombre virus (SN) as the etiologic agent of HPS (27, 41). Since then, HPS-associated viruses have been identified in 30 states, Canada, and South America (17, 25, 34, 37, 54). In contrast to HPS-associated strains, Prospect Hill (PH) is a distinct North American hantavirus which is not associated with any human disease (61).

There is a long history of HFRS derived from hantavirus infections. Hantaan virus (HTN), Seoul virus (SEO), Puumala virus (PUU), and Dobrava virus (DOB) are prominent causes of HFRS. HTN is the prototype HFRS-causing hantavirus and is the etiologic agent of Korean hemorrhagic fever (49). HTN is carried by the field mouse, *Apodemus agraricus*, and causes severe and sometimes fatal HFRS in humans. DOB is also carried by a discrete species of *Apodemus* (*A. flavicolus*) and causes severe HFRS, while SEO, carried by rats, and PUU, carried by bank voles, cause less severe forms of HFRS (49).

The means by which specific hantaviruses cause pulmonary or renal diseases is obscure (22, 27, 42, 64). In both animals and humans, hantavirus replication occurs predominantly in endothelial cells and macrophages (22, 27, 30, 42, 43, 62, 64). However, there is no evidence that hantaviruses cause disease while they persistently infect their primary small-mammal hosts (20, 49). In humans, hantaviruses cause acute pulmonary edema (HPS) (42, 64) or vascular hemorrhage and kidney dysfunction (HFRS), although both diseases are associated with acute thrombocytopenia and either disease may have pulmonary or renal components. However, there is little immune cell recruitment or damage to hantavirus-infected endothelial cells in either HPS or HFRS (42, 62, 64).

We have recently reported that β_3 integrins mediate the cellular entry of HPS-associated hantaviruses (SN and NY-1) (21). In contrast, the cellular entry of PH was blocked by antibodies to β_1- rather than β_3-specific integrins. We further demonstrated that SN and NY-1 entry occurred through an RGD tripeptide-independent integrin interaction. Although determinants of pathogenesis have not been defined for any hantavirus, unique integrin usage by HPS-associated hantaviruses and nonpathogenic PH suggested that integrin-specific interactions could contribute to the pathogenesis of HPS-associated hantaviruses.

Integrins are heterodimeric receptors composed of a combination of α and β subunits which mediate cell-cell adhesion, platelet aggregation, Ca^{2+} channel activation, and extracellular matrix protein (ECM) recognition (3–5, 23, 29, 31, 38, 39, 44, 51, 55, 59). Integrin-ligand interactions mediate the activa-

* Corresponding author. Mailing address: Departments of Medicine and of Molecular Genetics and Microbiology, SUNY at Stony Brook, HSC T17, Rm. 60, Stony Brook, NY 11794. Phone: (516) 444-2120. Fax: (516) 444-8886. E-mail: EMackow@mail.son.sunysb.edu.

tion and regulation of intracellular signaling pathways within cells, which further control both transcriptional and ligand binding cell functions (7, 14, 50, 52). Integrins are also linked to intracellular cytoskeletal elements which facilitate cellular migration on the ECM. ECM proteins such as vitronectin and fibronectin contain Arg-Gly-Asp (RGD) tripeptides, which are recognized by specific cellular integrins including $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_5\beta_1$ (55).

$\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins are abundant surface receptors of endothelial cells and platelets, respectively, and $\alpha_v\beta_3$ integrins are also present on macrophages (5, 29, 44, 51). $\alpha_{IIb}\beta_3$ integrins are present only on platelets, where they regulate platelet activation and participate in thrombus formation. $\alpha_v\beta_3$ integrins on endothelial cells also play central roles in maintaining capillary integrity through ligand binding interactions (5, 14, 28, 29, 36, 50, 55, 63). Further, $\alpha_v\beta_3$ and $\alpha_5\beta_1$ differentially regulate arteriolar smooth muscle, resulting in vasodilation or vasoconstriction, respectively, through the intracellular activation of calcium channels (3, 23, 38, 39, 57, 59).

Integrin usage and the requirements for the cellular entry of HFRS-causing hantaviruses have not been addressed and are likely to contribute to our understanding of HFRS pathogenesis. In this study, we investigated cell surface interactions which are required for the entry of HFRS viruses into endothelial, CHO, and Vero E6 cells. We demonstrated that ligands and antibodies to β_3 integrins block the cellular entry of HTN, SEO, and PUU and that recombinant β_3 integrins facilitate the entry of HFRS-causing hantaviruses into CHO cells. Our results indicate that β_3 integrins are central elements in the entry of pathogenic HPS- and HFRS-causing hantaviruses and implicate a common role for β_3 integrins in hantavirus pathogenesis.

MATERIALS AND METHODS

Cell and virus. Biosafety level 3 facilities were used throughout these experiments for hantavirus cultivation. Vero E6 and CHO cells were grown in Dulbecco's minimal essential medium supplemented with 10% fetal calf serum, L-glutamine, and penicillin-streptomycin (GIBCO). CHO cells were supplemented with 100 mM nonessential amino acids (GIBCO). Human umbilical vein endothelial cells (HUVECs) were grown in EBM-2 (Clonetics) supplemented with 0.1% endothelial cell growth factor. CHO cells stably transfected with integrins $\alpha_{IIb}\beta_3$ (CHO-A5) or $\alpha_v\beta_3$ (CHO-VNRC) were described previously (10, 35). HTN (76-118), SEO (SR-11), PUU (K-27), SN (CC107), NY-1, and PH were cultivated as previously described (11, 48, 49). SN and NY-1 are serotypically distinct hantaviruses associated with HPS (11, 21, 24, 48). PH has not been associated with human disease (61, 62).

Ligands, peptides, and antibodies. Vitronectin, fibronectin, laminin, fibrinogen, heparin, phytohemagglutinin, osteopontin, dextran sulfate, chondroitin sulfate A and B, and bovine serum albumin (BSA) were from Sigma. GRGDSP and GRGESP peptides were from GIBCO. Polyclonal rabbit antisera to β_1, β_3, β_4, α_1, α_2, α_5, and α_V, polyclonal goat antiserum to $\alpha_5\beta_1$ (blocking), and blocking monoclonal antibodies to β_2 (MAb1962) and $\alpha_v\beta_3$ (MAb1976) were from Chemicon. β_3-specific monoclonal antibodies M15 and LIBS were described previously (15, 16). Integrin-associated protein (IAP; CD47)-specific monoclonal antibodies B6H12 and 2D3 were described previously (18, 56). Rabbit sera and monoclonal antibody 7A12, specific for rotavirus proteins, were used as negative control sera in these studies (53). Antibodies were used at a range of concentrations (20 ng/ml to 50 μg/ml) in addition to those shown in the figures. After cells were washed, a 1:2,000 dilution of anti-mouse or anti-rabbit sera was incubated with the cells for 1 h at 37°C. N-protein-specific polyclonal rabbit serum has been described previously and was made to recombinant N-protein from NY-1 expressed in *Escherichia coli* (21). N-protein-specific sera cross-react with N-proteins from all tested hantaviruses (21a).

Ligand and antibody pretreatment of cells. Vero E6 cells or HUVECs were pretreated with antibodies or potentially competitive ligands for 1 h at 37°C. Antibodies (20 ng/ml to 50 μg/ml), ligands (1 to 50 μg/ml), and peptides (1 to 500 μg/ml) were preadsorbed to cells in 50 μl of phosphate-buffered saline (PBS)–1% BSA in duplicate wells of a 96-well plate. The sera or ligands were removed, and the monolayers were washed three times with PBS. Then 200 to 800 focus-forming units (FFU) of respective hantavirus inocula was adsorbed to the monolayers for 60 to 90 min. The viral inocula were removed, and the monolayers were washed three times and incubated 24 or 36 h prior to methanol fixation.

Quantitation of hantavirus-infected cells. Methods for immunoperoxidase staining of hantavirus antigens in infected cells were previously described (53). Briefly, cell monolayers were fixed in 100% methanol for 10 min at 4°C and incubated with polyclonal rabbit anti-nucleocapsid sera (1/2,000) for 1 h at 37°C. The monolayers were washed three to five times with PBS and incubated with a 1/5,000 dilution of a goat anti-rabbit horseradish peroxidase conjugate (Kirkegaard & Perry Laboratories). The monolayers were then washed three times with PBS and stained with 3-amino-9-ethylcarbazole (0.026%) in 0.1 M sodium acetate (pH 5.2)–0.03% H_2O_2 for 5 to 30 min (53). They were then washed once with distilled water, immunoperoxidase-stained infected cells were quantitated, and duplicate wells were compared. Quantitation of experimentally infected cells was compared to that of mock-infected or untreated infected cell controls.

RESULTS

To determine whether integrins or additional cell surface receptors mediate the cellular entry of HFRS-causing hantaviruses, we assessed the ability of pretreating cells with ligands to inhibit infection by HTN, SEO, PUU, and PH. A number of ligands to cell surface receptors were tested for their ability to block infectivity, including fibrinogen, heparin, vitronectin, fibronectin, osteopontin, laminin, phytohemagglutinin, dextran sulfate, and chondroitin sulfate A (5 ng/ml to 50 μg/ml). We found that SEO and PUU (Fig. 1B and C) infectivity was inhibited by >70% by vitronectin pretreatment whereas HTN infectivity (Fig. 1A) was reduced by just 60% following pretreatment with 40 μg of vitronectin per ml. HTN was partially inhibited (~20%) by fibronectin pretreatment, SEO infectivity was reduced slightly (~10%) by fibrinogen, and PUU infectivity was slightly reduced (20%) by osteopontin. In contrast, PH infectivity (Fig. 1D) was inhibited by >70% by fibronectin but was not blocked by vitronectin pretreatment (21). Preadsorption of other tested ligands (50 μg/ml) (see Materials and Methods) did not block HTN, SEO, PUU, or PH infectivity.

Vitronectin and fibronectin are ligands for specific cell surface integrins. To further analyze the involvement of cellular integrins in the entry of HFRS-causing hantaviruses, antibodies to specific integrins were preadsorbed to cells before they were infected with HTN, SEO, or PUU. Pretreatment of Vero E6 cells with antibodies to β_3 integrin subunits inhibited HTN, SEO, and PUU infectivity by >70% but had no effect on PH infectivity (<5%) (Fig. 2). In addition, antibodies to α_v integrin subunits blocked PUU by >70% but reduced the infectivity of SEO and PUU by only 50% and had no effect on PH. In contrast, α_5- and β_1-specific antibodies inhibited PH infectivity by ≥60% (Fig. 2).

In Fig. 3 polyclonal antibodies to $\alpha_v\beta_3$ or $\alpha_5\beta_1$ integrins were bound to Vero E6 cells prior to hantavirus adsorption. Similar to integrin subunit-specific sera, $\alpha_v\beta_3$-specific sera inhibited HTN, SEO, and PUU infectivity by approximately 70 to 80% while failing to block PH infectivity. Reciprocally, antisera to $\alpha_5\beta_1$ blocked PH infectivity (by >80%) but failed to inhibit HTN, SEO, or PUU infections. Vero E6 cells contain β_3 integrin subunits as demonstrated by Western blotting of cell lysates (data not shown). These results suggest that β_3-specific integrins mediate the cellular entry of HFRS-causing hantaviruses. Slight reductions in HFRS infectivity were also effected by some additional integrin ligands or α_v-specific sera with different hantaviruses (Fig. 3). This suggests that hantavirus interactions with cellular integrins are not identical even though they have common β_3 integrin involvement. Preadsorption of a mixture of sera (50 μg/ml each) which recognize $\alpha_5\beta_1$, $\alpha_v\beta_3$, β_2, and β_4 integrins did not further reduce the infectivity of any hantavirus (not shown).

$\alpha_v\beta_3$ integrins are present on the surface of endothelial cells infected by hantaviruses. To determine if $\alpha_v\beta_3$ integrins medi-

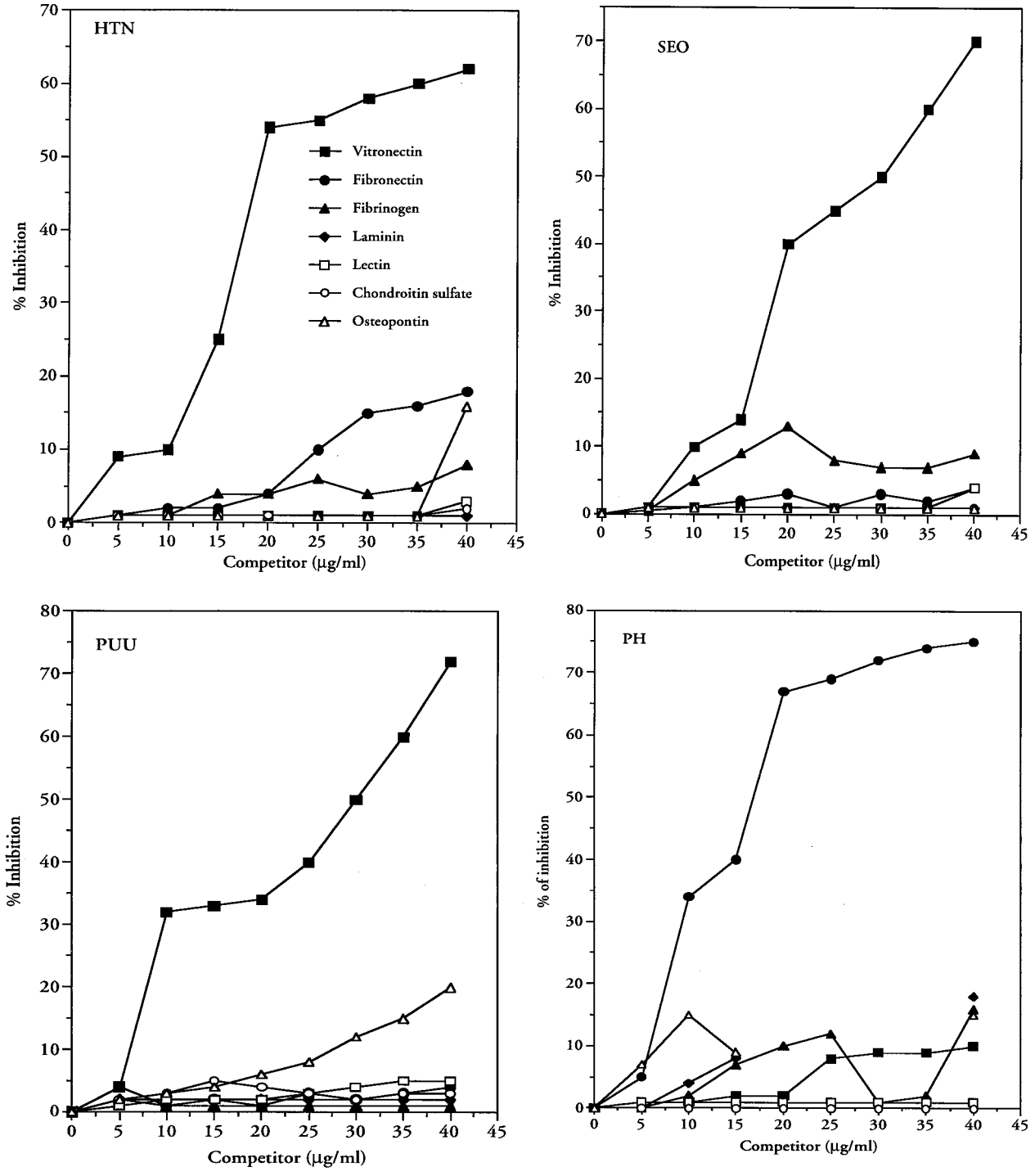

FIG. 1. Ligand-specific inhibition of hantavirus infectivity. Potentially competitive ligands (5 to 40 μg/ml, 50 μl) were preadsorbed to Vero E6 cells for 1 h prior to viral adsorption. Approximately 400 FFU of HTN, SEO, PUU, or PH was adsorbed to duplicate wells of a 96-well plate. Following adsorption, the inocula were removed and the cells were washed and further incubated for 24 to 36 h at 37°C in 5% CO_2 prior to methanol fixation. Hantavirus-infected cells were immunoperoxidase stained, as previously described (53), with polyclonal rabbit anti-nucleocapsid sera made to bacterially expressed and nickel affinity-purified NY-1 N-protein. Infected cells were quantitated and compared to cells with control infections without competitor proteins. The results were reproduced in at least three separate experiments and are presented as the percent inhibition with respect to control infections. Pretreatment with 1 to 500 μg of BSA per ml did not affect HTN, SEO, PUU, or PHV infectivity (results not shown).

ate the entry of HTN, SEO, PUU, or PHV into endothelial cells, we treated HUVECs with antibodies to integrin subunits prior to infection (Fig. 4). Both α_v- and β_3-specific sera reduced PUU infectivity by >70%, whereas HTN and SEO were inhibited >70% by only β_3-specific sera and approximately 40% by α_v-specific sera. In contrast, PH infectivity was inhibited by only β_1- and α_5-specific sera. This demonstrates that the infection of human endothelial cells by HFRS-causing hanta-

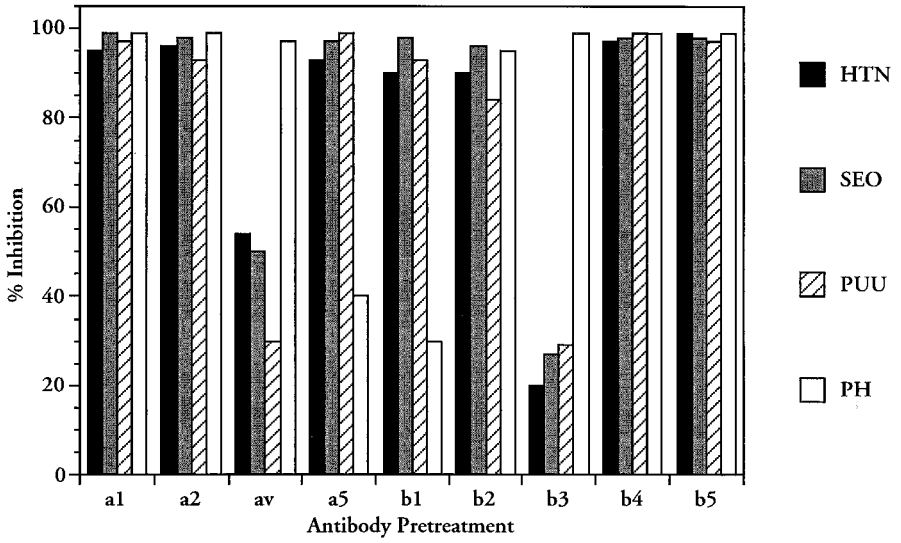

FIG. 2. Infectivity of HTN, SEO, PUU, and PH inhibited by integrin-specific antibodies. Duplicate wells of Vero E6 cells were pretreated for 1 h at 37°C with 20 μg of antibodies to specific integrins per ml, and after being washed with PBS they were similarly incubated with a 1/2,000 dilution of anti-rabbit or anti-mouse sera. The monolayers were washed, and HTN, SEO, PUU, or PH was adsorbed (Fig. 1). Infected cells were quantitated as in the experiment in Fig. 1. The number of FFU observed 36 h postinfection is expressed as a percentage of control infections for each viral inoculum. Polyclonal rabbit sera to β_1, β_3, β_4, α_1, α_2, α_5, α_V, and $\alpha_5\beta_1$ (blocking), as well as blocking monoclonal antibodies to β_2 (MAB1962) and $\alpha_v\beta_3$ (MAB1976), were from Chemicon.

viruses (HTN, SEO, PUU, and PH) is also mediated by specific integrin interactions.

To demonstrate that $\alpha_v\beta_3$ integrins are required for the cellular entry of HTN, SEO, and PUU, we studied the ability of recombinant integrins to mediate hantavirus infection of β_3 integrin-deficient CHO cells. CHO cell lines containing recombinant $\alpha_v\beta_3$ (CHO-VNRC) or $\alpha_{IIb}\beta_3$ (CHO-A5) (10, 35) integrins were infected with constant amounts of HTN, SEO, PUU, or PH. CHO-A5 or CHO-VNRC cells containing β_3-specific integrins dramatically enhanced the infectivity of HTN (Fig. 5), SEO, and PUU (results not shown) viruses but did not facilitate PH infections. Pretreatment of CHO cell lines with antisera to β_3 integrins specifically reduced the number of cells infected by HTN, SEO, or PUU by >90% (Fig. 5). A small number of infected CHO cells are observed following infection by these and other hantaviruses (21). These findings demonstrate that the introduction of β_3-specific integrins into cells facilitates HTN, SEO, and PUU infection but also suggests that additional cell surface interactions may contribute to hantavirus infectivity.

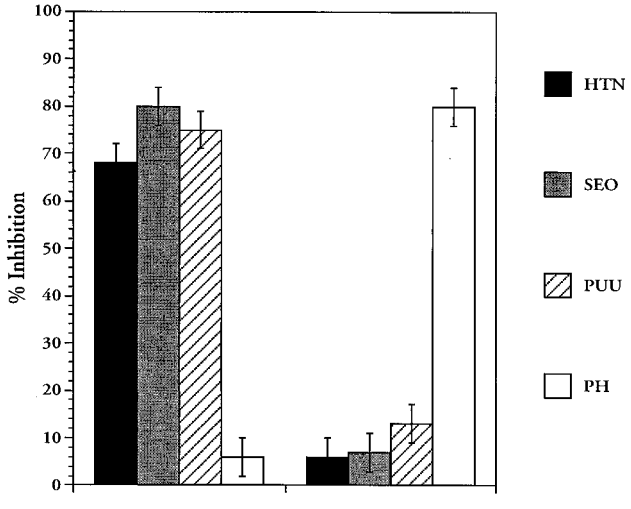

FIG. 3. HFRS hantavirus infectivity is inhibited by $\alpha_v\beta_3$-specific antibodies. Duplicate wells of Vero E6 cells were pretreated with 20 ng to 20 μg of $\alpha_v\beta_3$ or $\alpha_v\beta_3$ polyclonal rabbit sera per ml for 1 h at 37°C (results for 20 μg/ml are shown in the figure). The monolayers were washed three times with PBS, and a 1/2,000 dilution of anti-rabbit sera was incubated with cells for 1 h at 37°C. The monolayers were washed three times with PBS and infected with approximately 400 FFU of HTN, SEO, PUU, or PH for 1 h at 37°C. They were then washed and incubated for 24 h prior to immunoperoxidase staining of the hantavirus N-protein present in infected cells (see Materials and Methods) (Fig. 1). Infected cells were quantitated as in Fig. 1, and the results are presented as the percent inhibition with respect to control infections.

Cellular β_3 integrins are present in combination with the 50-kDa IAP, which is a cell surface receptor for thrombospondin (18, 19, 56). To determine if IAP-hantavirus interactions facilitate viral entry, we determined whether antibodies to IAPs were capable of inhibiting hantavirus infections. Prebinding either of two IAP-specific blocking monoclonal antibodies to cells had no effect on HTN infectivity (Fig. 6) and similarly did not alter SEO or PUU infectivity. In contrast, a β_3-specific monoclonal antibody inhibited HTN virus infectivity by >90%. Similarly, when both IAP-specific and β_3-specific antibodies were prebound to cells, no additional inhibition was observed over that due to β_3 antibody addition alone. These findings suggest that IAPs do not participate in additional hantavirus interactions with cells.

Integrin-ligand interactions are often mediated by RGD tripeptides. To determine if hantavirus-integrin interactions are RGD independent, we determined whether RGD- or RGE-containing peptides blocked HTN, SEO, or PUU infectivity. Preadsorbtion of RGD- or RGE-containing peptides to cells and coincubation of RGD or RGE peptides competitively during hantavirus adsorption had no effect on infectivity, even at concentrations as high as 500 μg/ml (Fig. 7). However, treatment of cells with RGD- but not RGE-containing peptides (20 μg/ml) prior to addition of vitronectin (10 μg/ml)

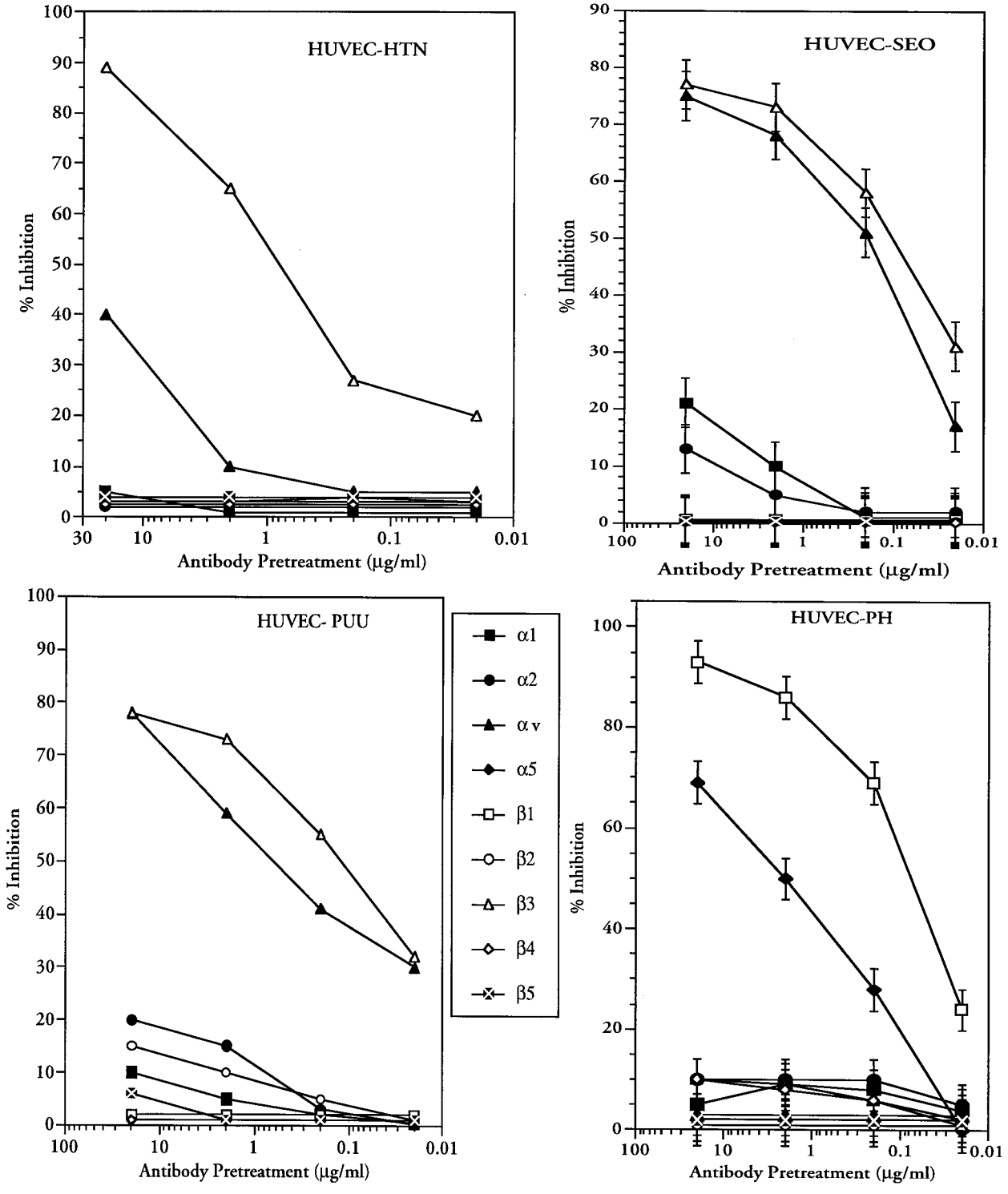

FIG. 4. Infection of HUVECs by HTN, SEO, PUU, and PH is inhibited by integrin-specific antibodies. HUVECs were pretreated with 20 ng to 20 μg of integrin-specific antibodies per ml for 1 h as described in the legend to Fig. 2. Following primary-antibody addition (for 1 h at 37°C) and PBS washing, a 1/2,000 dilution of anti-rabbit or anti-mouse sera, respectively, was incubated with the cells for 1 h at 37°C). HUVECs were subsequently infected with approximately 400 FFU of HTN, SEO, PUU, and PH for 1 h at 37°C. The monolayers were washed and incubated for 24 h prior to immunoperoxidase staining of the hantavirus N-protein present in infected cells (53). Infected cells were quantitated as in Fig. 1, and the results are presented as the percent inhibition with respect to control infections.

completely abolished the ability of vitronectin to inhibit HTN, SEO, or PUU infectivity. This is consistent with our finding that additional RGD-containing ligands failed to inhibit hantavirus infectivity. As a result, vitronectin is likely to block hantavirus infectivity through steric interference and not through a competitive RGD binding blockade. Similar to PH and HPS-associated hantaviruses (SN and NY-1), interactions of HTN, SEO, and PUU with integrins are RGD independent.

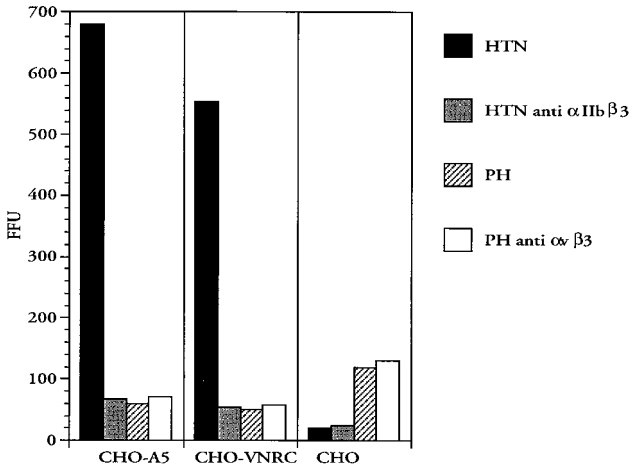

FIG. 5. Specificity of HTN and PH infection of CHO, CHO-A5, and CHO-VNRC cells. The number of infected cells following HTN or PH inoculation of CHO, CHO-A5, and CHO-VNRC cell lines is presented (10, 35). Inoculation with or without prior treatment of cells with 20 μg of rabbit anti-β3 polyclonal sera per ml was performed as in the experiment in Fig. 2. Duplicate wells of Vero E6 cells were pretreated for 1 h at 37°C with 20 μg of antibodies to specific integrins per ml and, after being washed with PBS, similarly incubated with a 1/2,000 dilution of anti-rabbit or anti-mouse sera. Monolayers were washed, and HTN or PH were subsequently adsorbed and quantitated 24 h postinfection as in Fig. 1.

c7E3 (ReoPro) is a commercially available $\alpha_v\beta_3$-specific humanized Fab fragment which is used therapeutically to inhibit thrombus formation in and around vascular stents (8, 9, 45). We tested the ability of c7E3 to inhibit the HTN, SEO, and PUU HFRS-causing strains as well as the NY-1 and SN HPS-associated strains and the nonpathogenic PH. Figure 8 demonstrates that c7E3 prebinding inhibited the infection of all hantaviruses which enter cells via interactions with $\alpha_v\beta_3$ but did not inhibit PH and further suggests a therapeutic potential for c7E3 or other $\alpha_v\beta_3$-specific antibody interventions for hantavirus disease.

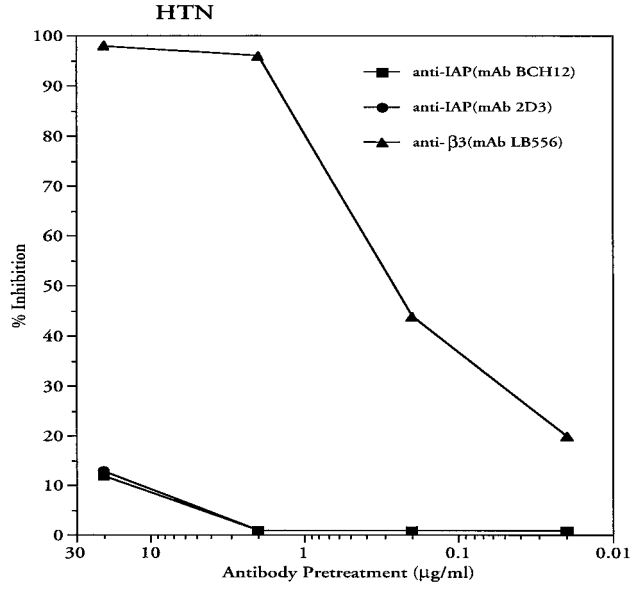

FIG. 6. HTN infectivity is not inhibited by antibodies to integrin-associated protein. Duplicate wells of Vero E6 cells were pretreated with 20 ng to 20 μg of monoclonal antibodies (2D3 and B6H12) to the 50-kDa IAP per ml for 1 h at 37°C or with LB556, which is specific for β_3 integrin subunits (18, 19, 56). The monolayers were washed three times with PBS, and a 1/2,000 dilution of anti-mouse sera was incubated with cells for 1 h at 37°C. Monolayers were washed three times with PBS and infected with approximately 400 FFU of HTN, SEO, PUU, or PH for 1 h at 37°C (results for HTN are shown). The monolayers were washed and incubated for 24 h at 37°C prior to immunoperoxidase staining of the hantavirus N-protein present in infected cells (53). Infected cells were quantitated as in Fig. 1, and the results are presented as the percent inhibition with respect to control infections. mAb, monoclonal antibody.

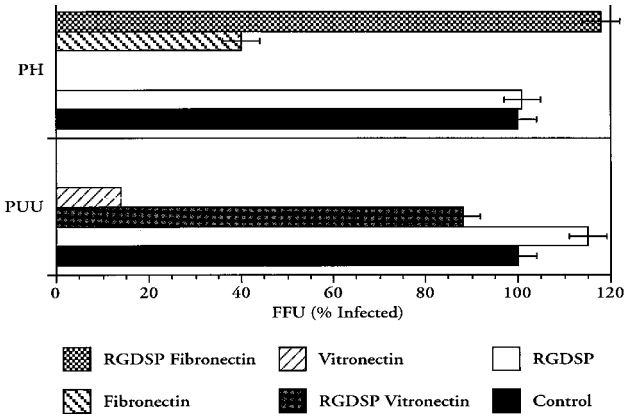

FIG. 7. HFRS integrin interactions are RGD independent. Vero E6 cells were pretreated with potentially competitive GRGDSP or GRGESP control peptides (≤500 μg/ml), vitronectin (20 μg/ml), or β_3-specific monoclonal antibody 15 (50 μg/ml) in 50 μl for 1 h at 37°C prior to viral adsorption. GRGDSP was added to the monolayers 60 min prior to addition of vitronectin or fibronectin and subsequent virus adsorption in GRGDSP-vitronectin or GRGDSP-fibronectin experiments. Following RGD pretreatments, approximately 400 FFU of HTN, SEO, PUU, or PH was adsorbed to duplicate wells of a 96-well plate (results for PUU are shown). Following adsorption, the inocula were removed and the cells were washed and further incubated for 24 to 36 h prior to methanol fixation. Hantavirus-infected cells were immunoperoxidase stained, as described previously (53), with polyclonal rabbit anti-nucleocapsid sera. Infected cells were quantitated and compared to control infections without competitor proteins. The results are presented as the percent inhibition with respect to control infections. BSA at all concentrations tested (1 to 100 μg/ml) did not affect HTN, SEO, PUU, or PHV infectivity (data not shown).

DISCUSSION

HFRS was first described over 50 years ago, and the first hantavirus was isolated in 1978 (33). A growing number of hantaviruses which cause human disease have recently been discovered throughout the world. DOB, Tula, Khabarovsk, and others are hantaviruses which have recently been identified in Eurasia (49). HPS-associated hantaviruses were discovered in North America in 1993 but have now been identified throughout the Americas (17, 25, 34, 37, 54). Interestingly, the first person-to-person transmission of a hantavirus (Andes) was recently reported and suggests that some hantaviruses have adopted new means of their transmission (12). These findings have increased interest in the interactions of hantaviruses with cells which mediate viral entry and provoke pathogenic responses.

In this report, we demonstrate that HFRS-causing hantaviruses, HTN, SEO, and PUU, gain cellular entry via specific $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrins. We previously demonstrated that the cellular entry of HPS-associated hantaviruses, SN and NY-1, also occurs through interactions with β_3 integrins (21). In contrast, entry of PH is not mediated by β_3 integrins and PH is not associated with any human disease (60, 62). Although the cellular entry of the few additional nonpathogenic hantavirus strains has yet to be studied, the ability of pathogenic HPS- and HFRS-causing hantaviruses to enter cells, via the same integrins, provides a compelling rationale for common pathogenic aspects of HFRS and HPS. Changes in hantavirus-integrin

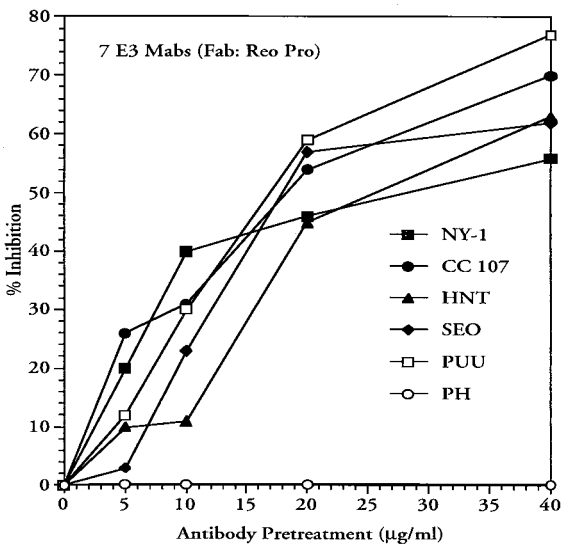

FIG. 8. Fab c7E3 (ReoPro) inhibits the infectivity of HPS- and HFRS-causing hantaviruses. Duplicate wells of Vero E6 cells were pretreated with 20 ng to 40 μg of mouse-human hybrid Fab, c7E3, to $\alpha_v\beta_3$ per ml for 1 h at 37°C. The monolayers were washed three times with PBS, and a 1/2,000 dilution of anti-mouse sera was incubated with the cells for 1 h at 37°C. The monolayers were washed three times with PBS and infected with approximately 400 FFU of HTN, SEO, PUU, NY-1, SN, and PH for 1 h at 37°C. They were then washed and incubated for 24 h prior to immunoperoxidase staining of the hantavirus N-protein present in infected cells (see Materials and Methods) (Fig. 1). Infected cells were quantitated as in Fig. 1, and the results are presented as the percent inhibition with respect to control infections.

usage or the use of additional cell surface receptors for viral entry could also participate in the transmission of hantaviruses among animal hosts and to humans.

Although it is possible that adapting hantaviruses to growth in Vero E6 cells selects for hantaviruses which gain entry via β_3 integrins, there are a number of reasons why this is very unlikely. We have now demonstrated that five separate hantavirus isolates, HTN, SEO, PUU, SN, and NY-1, with up to 60% divergent amino acid sequences in their surface glycoproteins enter cells via β_3 integrins and are effectively blocked by only one integrin ligand, vitronectin. Further, when the hantavirus genome was sequenced in its entireity from a patient, a small-mammal host, and Vero E6 cells after passage five times, no amino acid sequence differences were observed in any viral protein (6).

Our findings indicate that antibodies to integrins do not completely block hantavirus entry. However, anti-integrin antibodies do not completely inhibit adenovirus, papillomavirus, foot-and-mouth disease virus (FMDV), or coxsackievirus cellular entry (13, 26, 40, 46, 47, 58). Some of the residual binding (10%) reported for other viruses was observed to be nonsaturable and likely to be nonspecific (13). Uninhibitable hantavirus infectivity could similarly be due to some additional nonspecific interactions of hantaviruses with the cell surface or to additional specific hantavirus interactions which we have not yet identified. There is some indication of differential inhibitory effects on individual HFRS-causing hantaviruses by additional integrin-specific sera and ligands (Fig. 1 and 2). However, additive effects of α and β integrin antibodies or antibodies to $\alpha_v\beta_3$ integrins did not reduce the number of residually infected cells. In addition, IAP, which is a cell surface receptor present in tight conjunction with β_3 integrins (18, 19, 56), does not appear to mediate hantavirus entry, since antibodies to IAP had no effect on hantavirus infectivity in the presence or absence of anti-integrin antibodies. These findings do not alter our findings that the introduction of $\alpha_v\beta_3$-specific integrins into cells dramatically enhances cellular infection by HTN, SEO, and PUU and as a result that β_3 integrins confer the cellular entry of these HFRS-causing viruses.

Viruses which enter cells via $\alpha_v\beta_3$ integrins include adenoviruses, FMDV, and coxsackievirus A9, although adenoviruses and FMDV are also reported to use additional cell surface receptors (26, 40, 46, 47, 58). However, $\alpha_v\beta_3$ integrin usage by these viruses is dependent on integrin recognition of virally encoded RGD motifs at the ligand binding site (1, 26, 40, 46, 47, 58). RGD motifs are absent from all hantavirus proteins, and RGD peptides or additional RGD-containing integrin ligands fail to block infection by the hantaviruses which we have tested. Our findings demonstrate that HTN, SEO, and PUU (HFRS-causing hantaviruses), in addition to previously reported HPS-associated hantaviruses, associate with $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins through unique RGD-independent interactions (21). This further suggests that vitronectin sterically blocks hantavirus-integrin interactions and that hantaviruses interact with unique integrin regions or require more complex cell receptor interactions for cellular entry.

Hantaviruses predominantly infect endothelial cells and macrophages and impact platelet functions. β_3 integrins are present on macrophages and are prominent endothelial-cell and platelet receptors. Integrins bind extracellular matrices; specify cell-cell adherence of platelets, macrophages, and endothelial cells; and mediate endothelial-cell migration (29, 55). β3 integrins also activate specific intracellular signaling pathways within platelets and endothelial cells, which further regulate a variety of adherence functions as well as intracellular calcium currents (2, 7, 32, 52, 59).

β_3 integrins play key roles in maintaining vascular integrity through platelet and endothelial cell barrier functions. Additionally, $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins on smooth muscle cells mediate vasodilation and vasoconstriction of arterioles, respectively, through intracellular signaling pathways linked to L-type Ca^{2+} channels (2, 32, 38, 39, 59). Thus, $\alpha_v\beta_3$- and $\alpha_5\beta_1$-specific integrins differentially regulate blood flow and vascular tone (59). Interestingly, nonpathogenic PH and pathogenic hantaviruses are linked to the use of integrins which effect opposite calcium currents in endothelial and smooth muscle cells (2, 32, 38, 39, 59). The differential linkage of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins to calcium currents in endothelial and smooth muscle cells provides a further rationale for the correlation of integrin usage and hantavirus pathogenesis.

Since hantaviruses alter vascular permeability and cause thrombocytopenia, hantavirus interactions with β_3 integrins could participate in common pathogenic HPS and HFRS responses. It remains to be addressed whether hantavirus interactions with β_3 integrins cause thrombocytopenia or alter endothelial-cell permeability. However, the regulatory functions of β_3 integrins in both platelet activation and endothelial-cell barrier function are clear and provide plausible means for hantavirus-protein interactions to mediate endothelial-cell- and platelet-specific vascular changes. The presence of large differences in the glycoprotein composition of HFRS- and HPS-causing hantaviruses (approximately 60 and 40% unique residues in the G1 and G2 proteins, respectively) further suggests how differential pathogenic responses could be effected by individual viruses despite common β_3 integrin usage.

Hantavirus interactions with $\alpha_v\beta_3$ integrins provide a clear means of altering vascular permeability during infection and also provide a potential point for therapeutic intervention during hantavirus infections. ReoPro is a human-mouse hybrid

Fab fragment, c7E3, which recognizes both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ integrins and is used therapeutically to inhibit platelet aggregation (8, 9, 45). We tested the ability of c7E3 to block the infectivity of both HFRS- and HPS-associated hantaviruses and demonstrated that it is capable of inhibiting these hantaviruses (Fig. 8). These findings suggest that c7E3 should be considered for the therapeutic treatment of hantavirus diseases. However, blocking platelet aggregation during hantavirus infection could also have negative effects on thrombocytopenic patients. Antibodies which block hantavirus-integrin interactions without altering platelet activation may provide less complicated and more specific intervention for hantavirus infection.

The divergence of hantavirus surface glycoproteins and common β_3 integrin usage provides further insight into the interaction of hantaviruses with cells. Differences in the respective G1 and G2 glycoproteins of hantaviruses are substantial, and this variation is likely to be associated with additional interactions which contribute to unique pathogenic responses to individual viruses. β_3 integrin usage also suggests that common elements exist on G1 or the more highly conserved G2 surface glycoprotein which mediate viral attachment to integrins. Although there is currently no data defining the virion attachment protein, the development of antibodies which recognize the hantavirus attachment protein and block integrin interactions are of interest since they are likely to provide an additional point for therapeutic intervention and vaccine development.

ACKNOWLEDGMENTS

We thank Dmitry Goldgaber for stimulating discussions and encouragement in pursuing these studies. We thank Connie Schmaljohn for providing HTN, SEO, PUU, SN, and PUU; Barry Coller for providing ReoPro for these experiments; and Robert Shope for providing PH.

This work was supported by Merit and VA/DOD Awards from the Veterans Administration and by NIH grants R03AI42150 (E.R.M.), R01-AI31016 (E.R.M.), HL48728 (M.H.G.), and AR27214 (M.H.G.).

REFERENCES

1. **Berinstein, A., M. Roivainen, T. Hovi, P. W. Mason, and B. Baxt.** 1995. Antibodies to the vitronectin receptor (integrin alpha V beta 3) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. J. Virol. **69:**2664–2666.
2. **Bilato, C., K. A. Curto, R. E. Monticone, R. R. Pauly, A. J. White, and M. T. Crow.** 1997. The inhibition of vascular smooth muscle cell migration by peptide and antibody antagonists of the alphavbeta3 integrin complex is reversed by activated calcium/calmodulin-dependent protein kinase II. J. Clin. Investig. **100:**693–704.
3. **Bossu, J. L., A. Feltz, J. L. Rodeau, and F. Tanzi.** 1989. Voltage-dependent transient calcium currents in freshly dissociated capillary endothelial cells. FEBS Lett. **255:**377–380.
4. **Butcher, E., and L. Picker.** 1996. Lymphocyte homing and homeostasis. Science. **272:**60–66.
5. **Cheresh, D.** 1987. Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc. Natl. Acad. Sci. **84:**6471–6475.
6. **Chizhikov, V., C. Spiropoulou, S. Morzunov, M. Monroe, C. Peters, and S. Nichol.** 1995. Complete genetic characterization and analysis of isolation of Sin Nombre virus. J. Virol. **69:**8132–8136.
7. **Clark, E. A., S. J. Shattil, M. H. Ginsberg, J. Bolen, and J. S. Brugge.** 1994. Regulation of the protein tyrosine kinase pp72syk by platelet agonists and the integrin alpha IIb beta 3. J. Biol. Chem. **269:**28859–28864.
8. **Coller, B.** 1997. GPIIb/IIIa antagonists: pathophysiologic and therapeutic insights from studies of c7E3 Fab. Thromb. Haemostasis **78:**730–735.
9. **Coller, B.** 1997. Platelet GPIIb/IIIb antagonists: the first anti-integrin receptor therapeutics. J. Clin. Investig. **99:**1467–1471.
10. **Diaz-Gonzalez, F., J. Forsyth, B. Steiner, and M. Ginsberg.** 1996. Trans-dominant inhibition of integrin function. Mol. Biol. Cell **7:**1939–1951.
11. **Elliott, L. H., T. G. Ksiazek, P. E. Rollin, C. F. Spiropoulou, S. Morzunov, M. Monroe, C. S. Goldsmith, C. D. Humphrey, S. R. Zaki, J. W. Krebs, et al.** 1994. Isolation of the causative agent of hantavirus pulmonary syndrome. Am. J. Trop. Med. Hyg. **51:**102–108.
12. **Enria, D., P. Padula, E. L. Segura, N. Pini, A. Edelstein, C. R. Posse, and M. C. Weissenbacher.** 1996. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. Medicina **56:**709–711.
13. **Evander, M., I. Frazer, E. Payne, Y. Qi, K. Hengst, and N. McMillan.** 1997. Identification of the a6 integrin as a candidate receptor for papillomaviruses. J. Virol. **71:**2449–2456.
14. **Faull, R. J., and M. H. Ginsberg.** 1996. Inside-out signaling through integrins. J. Am. Soc. Nephrol. **7:**1091–1097.
15. **Frelinger, A. L., III, I. Cohen, E. F. Plow, M. A. Smith, J. Roberts, S. C. Lam, and M. H. Ginsberg.** 1990. Selective inhibition of integrin function by antibodies specific for ligand-occupied receptor conformers. J. Biol. Chem. **265:**6346–6352.
16. **Frelinger, A. L., III, X. P. Du, E. F. Plow, and M. H. Ginsberg.** 1991. Monoclonal antibodies to ligand-occupied conformers of integrin alpha IIb beta 3 (glycoprotein IIb-IIIa) alter receptor affinity, specificity, and function. J. Biol. Chem. **266:**17106–17111.
17. **Fulhorst, C. F., M. C. Monroe, R. A. Salas, G. Duno, A. Utrera, T. G. Ksiazek, S. T. Nichol, N. M. de Manzione, D. Tovar, and R. B. Tesh.** 1997. Isolation, characterization and geographic distribution of Caño Delgadito virus, a newly discovered South American hantavirus (family Bunyaviridae). Virus Res. **51:**159–171.
18. **Gao, A. G., F. P. Lindberg, J. M. Dimitry, E. J. Brown, and W. A. Frazier.** 1996. Thrombospondin modulates alpha v beta 3 function through integrin-associated protein. J. Cell Biol. **135:**533–544.
19. **Gao, A. G., F. P. Lindberg, M. B. Finn, S. D. Blystone, E. J. Brown, and W. A. Frazier.** 1996. Integrin-associated protein is a receptor for the C-terminal domain of thrombospondin. J. Biol. Chem. **271:**21–24.
20. **Gavrilovskaya, I., N. Apekina, A. Bernshtein, V. Demina, N. Okulova, Y. Myasnikov, and M. Chumakov.** 1990. Pathogenesis of hemorrhagic fever with renal syndrome virus infection and mode of horizontal transmission of hantavirus in bank voles. Arch. Virol. Suppl **1:**57–62.
21. **Gavrilovskaya, I. N., M. Shepley, R. Shaw, M. H. Ginsberg, and E. R. Mackow.** 1998. B3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc. Natl. Acad. Sci. USA **95:**7074–7079.

21a. **Gavrilovskaya, I., R. La Monica, M.-E. Fay, B. Hjelle, C. Schmaljohn, R. Shaw, and E. R. Mackow.** 1999. New York 1 and Sin Nombre viruses are serotypically distinct viruses associated with hantavirus pulmonary syndrome. J. Clin. Microbiol. **37:**122–126.

22. **Gonzalez-Scarano, F., and N. Nathanson.** 1996. Bunyaviridae, p. 1473–1504. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Virology, 3rd ed, vol. 1. Lippincott-Raven, Philadelphia, Pa.
23. **Hawiger, J.** 1995. Mechanisms involved in platelet vessel wall interaction. Thromb. Haemostasis **74:**369–372.
24. **Hjelle, B., S. W. Lee, W. Song, N. Torrez-Martinez, J. W. Song, R. Yanagihara, I. Gavrilovskaya, and E. R. Mackow.** 1995. Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse, *Peromyscus leucopus*: genetic characterization of the M genome of New York virus. J. Virol. **69:**8137–8141.
25. **Hjelle, B., N. Torrez-Martinez, and F. T. Koster.** 1996. Hantavirus pulmonary syndrome-related virus from Bolivia. Lancet **347:**57.
26. **Huang, S., T. Kamata, Y. Takada, Z. Ruggeri, and G. Nemerow.** 1996. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. **70:**4502–4508.
27. **Hughes, J. M., C. J. Peters, M. L. Cohen, and B. W. Mahy.** 1993. Hantavirus pulmonary syndrome: an emerging infectious disease. Science. **262:**850–851.
28. **Huttenlocher, A., M. H. Ginsberg, and A. F. Horwitz.** 1996. Modulation of cell migration by integrin-mediated cytoskeletal linkages and ligand-binding affinity. J. Cell Biol. **134:**1551–1562.
29. **Hynes, R.** 1992. Integrins: versatility, modulation and signaling in cell adhesion. Cell **69:**11–25.
30. **Khan, A. S., R. F. Khabbaz, L. R. Armstrong, R. C. Holman, S. P. Bauer, J. Graber, T. Strine, G. Miller, S. Reef, J. Tappero, P. E. Rollin, S. T. Nichol, S. R. Zaki, R. T. Bryan, L. E. Chapman, C. J. Peters, and T. G. Ksiazek.** 1996. Hantavirus pulmonary syndrome: the first 100 US cases. J. Infect. Dis. **173:**1297–1303.
31. **Lampugnani, M., M. Resnati, E. Dejana, and P. Marchisiio.** 1991. The role of integrins in maintenance of endothelial monolayer integrity. J. Cell Biol. **112:**479–490.
32. **Leavesley, D., M. Schwartz, M. Rosenfeld, and D. Cheresh.** 1993. Integrin b1 and b3-mediated endothelial cell migration is triggered through distinct signaling mechanisms. J. Cell Biol. **121:**163–170.
33. **Lee, H., P. Lee, and K. Johnson.** 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. **137:**298–308.
34. **Levis, S., J. Rowe, S. Morzunov, D. Enria, and S. St. Jeor.** 1997. New Hantaviruses causing hantavirus pulmonary syndrome in central America. Lancet **349:**998–999.
35. **Loftus, J., T. O'Toole, E. Plow, A. Glass, A. Frelinger III, and M. Ginsberg.** 1990. A B3 integrin mutation abolishes ligand binding and alters divalent cation-dependent conformation. Science **249:**915–918.
36. **Loftus, J., J. Smith, and M. Ginsberg.** 1994. Integrin-mediated cell adhesion: the extracellular face. J. Biol. Chem. **269:**25235–25238.
37. **Lopez, N., P. Padula, C. Rossi, M. E. Lazaro, and M. T. Franze-Fernandez.** 1996. Genetic identification of a new hantavirus causing severe pulmonary

syndrome in Argentina. Virology **220:**223–226.
38. **Mogford, J. E., G. E. Davis, and G. A. Meininger.** 1997. RGDN peptide interaction with endothelial alpha5beta1 integrin causes sustained endothelin-dependent vasoconstriction of rat skeletal muscle arterioles. J. Clin. Investig. **100:**1647–1653.
39. **Mogford, J. E., G. E. Davis, S. H. Platts, and G. A. Meininger.** 1996. Vascular smooth muscle alpha v beta 3 integrin mediates arteriolar vasodilation in response to RGD peptides. Circ Res. **79:**821–826.
40. **Neff, S., D. Sa-Carvalho, E. Rieder, P. W. Mason, S. D. Blystone, E. J. Brown, and B. Baxt.** 1998. Foot-and-mouth disease virus virulent for cattle utilizes the integrin alpha(v)beta3 as its receptor. J. Virol. **72:**3587–3594.
41. **Nichol, S. T., C. F. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, and C. J. Peters.** 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science. **262:**914–917.
42. **Nolte, K. B., R. M. Feddersen, K. Foucar, S. R. Zaki, F. T. Koster, D. Madar, T. L. Merlin, P. J. McFeeley, E. T. Umland, and R. E. Zumwalt.** 1995. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. Hum. Pathol. **26:**110–120.
43. **Pensiero, M. N., J. B. Sharefkin, C. W. Dieffenbach, and J. Hay.** 1992. Hantaan virus infection of human endothelial cells. J. Virol. **66:**5929–5936.
44. **Phillips, D., I. Charo, and L. Parise.** 1988. The platelet membrane glycoprotein GPIIb/IIIa complex. Blood. **71:**831–843.
45. **Reverter, J., S. Beguin, H. Kessels, R. Kumar, H. Hemler, and B. Coller.** 1996. Inhibition of Platelet-mediated, tissue factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody. J. Clin. Investig. **98:**863–874.
46. **Roivainen, M., T. Hyypia, L. Piirainen, N. Kalkkinen, G. Stanway, and T. Hovi.** 1991. RGD-dependent entry of coxsackievirus A9 into host cells and its bypass after cleavage of VP1 protein by intestinal proteases. J. Virol. **65:** 4735–4740.
47. **Roivainen, M., L. Piirainen, T. Hovi, I. Virtanen, T. Riikonen, J. Heino, and T. Hyypia.** 1994. Entry of coxsackievirus A9 into host cells: specific interactions with alpha v beta 3 integrin, the vitronectin receptor. Virology **203:** 357–365.
48. **Schmaljohn, A. L., D. Li, D. L. Negley, D. S. Bressler, M. J. Turell, G. W. Korch, M. S. Ascher, and C. S. Schmaljohn.** 1995. Isolation and initial characterization of a newfound hantavirus from California. Virology **206:** 963–972.
49. **Schmaljohn, C., and B. Hjelle.** 1997. Hantaviruses: a global disease problem. Emerging Infect. Dis. **3:**95–104.
50. **Schwartz, M., M. Shaller, and M. Ginsberg.** 1995. Integrins: emerging paradigms of signal transduction. Annu. Rev. Cell Dev. Biol. **11:**549–599.
51. **Sepp, N. T., L. J. Li, K. H. Lee, E. J. Brown, S. W. Caughman, T. J. Lawley, and R. A. Swerlick.** 1994. Basic fibroblast growth factor increases expression of the alpha v beta 3 integrin complex on human microvascular endothelial cells. J. Investig. Dermatol. **103:**295–299.
52. **Shattil, S. J., M. H. Ginsberg, and J. S. Brugge.** 1994. Adhesive signaling in platelets. Curr. Opin. Cell Biol. **6:**695–704.
53. **Shaw, R. D., P. T. Vo, P. A. Offit, B. S. Coulson, and H. B. Greenberg.** 1986. Antigenic mapping of the surface proteins of rhesus rotavirus. Virology **155:**434–451.
54. **Song, J. W., L. J. Baek, D. C. Gajdusek, R. Yanagihara, I. Gavrilovskaya, B. J. Luft, E. R. Mackow, and B. Hjelle.** 1994. Isolation of pathogenic hantavirus from white-footed mouse (*Peromyscus leucopus*). Lancet **344:** 1637. (Letter.)
55. **Sugimori, T., D. Griffith, and M. Arnaout.** 1997. Emerging paradigms of integrin ligand binding and activation. Kidney Int. **51:**1454–1462.
56. **Ticchioni, M., M. Deckert, F. Mary, G. Bernard, E. J. Brown, and A. Bernard.** 1997. Integrin-associated protein (CD47) is a comitogenic molecule on CD3- activated human T cells. J. Immunol. **158:**677–684.
57. **Tsukada, H., X. Ying, C. Fu, S. Ishikawa, P. McKeown-Longo, S. Albelda, S. Bhattacharya, B. A. Bray, and J. Bhattacharya.** 1995. Ligation of endothelial alpha v beta 3 integrin increases capillary hydraulic conductivity of rat lung. Circ. Res. **77:**651–659.
58. **Wickham, T. J., P. Mathias, D. A. Cheresh, and G. R. Nemerow.** 1993. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. Cell **73:**309–319.
59. **Wu, X., J. E. Mogford, S. H. Platts, G. E. Davis, G. A. Meininger, and M. J. Davis.** 1998. Modulation of calcium current in arteriolar smooth muscle by alphavbeta3 and alpha5beta1 integrin ligands. J. Cell Biol. **143:**241–252.
60. **Yanagihara, R., H. L. Amyx, P. W. Lee, D. M. Asher, C. J. Gibbs, and D. C. Gajdusek.** 1988. Experimental hantavirus infection in nonhuman primates. Arch. Virol. **101:**125–130.
61. **Yanagihara, R., C. A. Daum, P. W. Lee, L. J. Baek, H. L. Amyx, D. C. Gajdusek, and C. J. Gibbs.** 1987. Serological survey of Prospect Hill virus infection in indigenous wild rodents in the USA. Trans. R. Soc. Trop. Med. Hyg. **81:**42–45.
62. **Yanagihara, R., and D. J. Silverman.** 1990. Experimental infection of human vascular endothelial cells by pathogenic and nonpathogenic hantaviruses. Arch. Virol. **111:**281–286.
63. **Ylanne, J., J. Huuskonen, T. E. O'Toole, M. H. Ginsberg, I. Virtanen, and C. G. Gahmberg.** 1995. Mutation of the cytoplasmic domain of the integrin beta 3 subunit. Differential effects on cell spreading, recruitment to adhesion plaques, endocytosis, and phagocytosis. J. Biol. Chem. **270:**9550–9557.
64. **Zaki, S., P. Greer, L. Coffield, C. Goldsmith, K. Nolte, K. Foucar, R. Feddersen, R. Zumwalt, G. Miller, P. Rollin, T. Ksiazek, S. Nichol, and C. Peters.** 1995. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. Am. J. Pathol. **146:**552–579.